during EADs; and 5) the transition from phase-2 to phase-3 EADs. We show that: 1) increasing RyR leakiness and/or SR Ca uptake rate promotes DADs, but can either suppress or potentiate EADs; 2) EADs can occur after a long plateau phase, and random opening of L-type Ca channels in plateau voltage prevents Ca waves and thus Ca oscillations in the long plateau phase in which both cytosolic and SR Ca remain high; 3) two distinct DAD mechanisms exist_a non-Ca wave mediated spontaneous SR Ca release and Ca wave-mediated spontaneous SR Ca release; and 4) Ca accumulation under Ca overload conditions can cause a transition from phase-2 EADs to phase-3 EADs. These simulations not only recapitulated most of the known complex EAD and DAD dynamics but also allowed the underlying ionic and dynamical mechanisms to be analyzed, providing novel insights into these arrhythmogenic AP dynamics.

1324-Pos Board B275

Structural and Functional Defects of T-Tubular System and Their Implications in Calcium Release and Contraction in a Mouse Model of Hypertrophic Cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is the most prevalent monogenic heart disorder and is determined by mutation of sarcomeric genes including cardiac troponin T (cTnT). Delayed relaxation and diastolic dysfunction are the main determinants of symptoms in patients. Here we use a mouse model of HCM carrying the clinically-relevant Δ160E cTnT mutation to assess isometric twitch tension from ventricular trabeculae. As compared to wild type siblings, Δ160E mice display prolonged kinetics of both force development and relaxation, blunted force frequency response with reduced active tension at high stimulation frequency, and increased occurrence of spontaneous contractions. The role of T-tubular defects in determining contraction abnormalities has been investigated using a multi-photon random access microscopy to dissect the spatio-temporal relationship between T-tubular electrical activity and Ca^{2+} release in isolated cardiomyocytes. We found a significant number of tubular elements failing in propagating AP with correspondent delay of local Ca^{2+} release. At variance with wild type we also observe increased beat-to-beat variability of Ca^{2+} rise as well as higher spark frequency. In Δ160E mice, electro-mechanical defects are associated with structural and ultra-structural alteration assessed by confocal and electron microscopy. Although T-tubular density and regularity are both significantly reduced in mutant the entity of such structural changes is modest and can hardly account for the observed functional alterations. Transmission electron microscopy reveals additional ultra-structural alterations spanning the Z-line, potentially contributing to non-homogeneous Ca^{2+} release and contractile dysfunction.

1325-Pos Board B276

Simultaneous Detection and Colocalization of Calcium Sparks and Ryanodine Receptor Clusters in Cardiac Myocytes

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Further understanding of calcium handling and excitation-contraction coupling in cardiac myocytes requires quantitative data analysis methods to characterize calcium release events in terms of structural properties of the cell. Such automatic methods provide a robust, consistent and reproducible characterization of physiological systems from observed experimental data. We present a multilevel analysis that simultaneously focuses on both the occurrence of spontaneous calcium sparks and the distribution of RyR clusters across a cardiac myocyte. The approach localizes the release events and determines the distance to the nearest ryanodine receptor cluster, therefore providing quantitative information on the spatio-temporal distribution of activation sites in the cell. The location of clusters takes into account motion artifacts produced by calcium waves or mini-waves. The method has been validated with line-scan confocal microscopy data from mouse ventricular myocytes and provides a full characterization of the spark morphology including its amplitude, baseline, decay time, upstroke time, Full-Width at Half-Maximum, Full-Duration at Half-Maximum and background noise.
The processing is applied to linescan images of both RyR clusters and calcium fluorescence. It includes the following steps: i) Adaptive filtering of background fluorescence fluctuations using a robust estimation of the noise by means of the median absolute deviation of the basal fluorescence signal. ii) Individual sparks are detected by using a modified watershed segmentation method that includes stopping rules for both event size and shape. iii) Location of RyR clusters was implemented by thresholding a continuous wavelet transform of the cluster image (robust to motion and contraction). iV) Measurement of spark morphology properties, including the distance from each spark to the closest RyR cluster.

1326-Pos Board B277

Reduced Ca Flux via Ryanodine Receptor Cluster Size or Unitary Current can both Promote Long-Lasting Ca Sparks

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Calcium (Ca) sparks are fundamental E-C coupling events with typical duration of 10-50 ms. Long-lasting Ca release events, lasting >10 times longer than typical Ca sparks, have been observed experimentally when ryanodine receptors (RyRs) are partially blocked by tetracaine or ruthenium red (Zima et al., 2008 BJ & Circ Res). However, the mechanism of these events is not fully understood. Here, we use a physiologically detailed mathematical model of subcellular Ca cycling, and show how RyR cluster size (or 'effective' cluster size when RyRs are partially blocked) can affect long-lasting Ca release events. The single cluster contains a few to several hundred RyRs, and we use a 4-state Markov model of the RyR. Each RyR opens stochastically and is regulated by cytosolic and luminal Ca. The number of RyR channels in the cluster, diffusion within the SR network, diffusion between network and junctional SR, SERCA uptake rate and RyR open probability were varied. In order for long-lasting release events, opening events within the cluster must occur continuously since the typical open time of the RyR is only a few milliseconds. We found (1) if the number of RyRs is too small, it is difficult to keep consecutive openings and stochastic attrition terminates the release, (2) if the number of RyRs is too large, the depletion of Ca from the junctional SR terminates the release, and (3) the involvement of moderate sized RyR clusters (~8; or reduced flux/RyR) allows stable release flux lasting >500 ms, wherein local [Ca]SR can be maintained by intra-SR diffusion.

1327-Pos Board B278

Expression and Function of Inositol 1,4,5-Trisphosphate Receptors in the Heart

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Calcium is a second messenger that is essential for the control of a variety of cellular functions. In the heart, calcium plays an integral role in many cellular processes including muscle contraction, gene expression, and cell death. The inositol 1,4,5-trisphosphate receptor (IP_3R) is a calcium channel that is ubiquitously expressed throughout the body. There are three IP_3R isoforms encoded by separate genes. All three IP_3R isoforms are expressed in the heart, with IP_3R-2 reported to being most predominant with regards to both expression levels and functional significance. The role of IP_3R signaling in the heart has been controversial in part due to its low expression level compared to other calcium channels. However, several groups have shown that IP_3R-2 may play a key role in the progression of hypertrophy and apoptosis. The functional roles of IP_3R-1 and IP_3R-3 in the heart are essentially unexplored despite measureable expression levels. We hypothesize that all three IP_3R isoforms can contribute to calcium transients and cardiac stress induced by endothelin-1 (ET-1). As our models we used embryonic mouse ventricular cardiomyocytes and the H9C2 cell line. Contrary to previous reports, we found that there is similar level of expression of all three IP_3Rs in mouse ventricular cardiomyocytes and H9C2 cells. We next treated H9C2 cells with ET-1 for up to 96 hours and compared RNA and protein levels to controls. We found that was similar protein and RNA levels of all three IP_3R isoforms in H9C2 cells after 48, 72 and 96 hours treatment with ET-1. These results demonstrate that all three IP_3R isoforms are expressed in the heart at similar levels. Our results also suggest that ET-1 does not significantly affect IP_3R expression levels, with possible significance for cardiac hypertrophy.